

The Species Concept in Pteridophyta with Special Reference to *Isoëtes*

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The definition, description, and identification of *Isoëtes* species has always been difficult. This is primarily the result of an overall morphological simplicity and extreme phenotypic plasticity that combine to reduce the number of stable and therefore diagnostic characters. Such aspects of the biology of *Isoëtes* have, historically, resulted in rather diverse specific treatments and circumscriptions. Recent studies in the genus have tended to emphasize previously overlooked characters and to apply both modern experimental techniques and the principles of population genetics in order to resolve many of these inconsistencies. Investigations utilizing cytological data, germination and hybridization studies, and protein electrophoresis have shown that over the range of a species its boundaries vary due to clinal variation, non-patterned geographical variation, interspecific hybridization, and the production of fertile allopolyploids. Such approaches have greatly increased our understanding of the dynamics of this genus.

At the outset, it should be stated that it is not the objective of this paper to discuss the theoretical implications, or the limitations and benefits of the various species concepts that are currently in existence. Such discussions are numerous and can be found throughout the pages of many of the major systematic journals such as *Systematic Botany*, *Systematic Zoology* and *Biology and Philosophy*. Rather, this paper is designed to illustrate both the current and the historical application of the various species concepts in pteridophytes with particular reference to *Isoëtes*. It is hoped that the reader will come away with an appreciation of the progressive nature involved in the elucidation of species and for the (practical) necessity of applying multiple species concepts during any given systematic inquiry (see also Zander, 1985). To illustrate such processes the following examples will be presented: the *I. storkii* aggregate of Costa Rica; the *I. melanopoda* complex of the southeastern United States; and the *Isoëtes* species of northeastern North America. As a prelude to these examples, a brief review of the species concept and a discussion of the role of herbarium material will be presented.

Species concepts.—For the purposes of this paper, the species concepts of consequence are: the typological species concept (TSC), the morphological species concept (MSC) and the biological species concept (BSC). The TSC is associated with all poorly known groups. Typically it is associated with rare or at least rarely collected species and is almost always employed when only a single collection is known for the species; i.e., simply, the species is circumscribed upon the only materials available—the type. The MSC, the traditional concept of

most herbarium workers, is broader in scope and relies upon morphological hiatuses between individuals and/or populations in defining species. This concept incorporates the variation known from as many populations as possible, and generally includes field observations. Under the framework of the MSC the herbarium taxonomist frequently employs extrapolations from better known taxa to justify the inclusion of somewhat disparate elements into a single species (e.g., Tryon, 1955, p. 3). The BSC is dependent not on morphological boundaries but on reproductive ones. The systematist employing the BSC defines and describes interbreeding populations as the basic taxonomic unit.

The role of herbarium material.—A major factor involved in the transition from a TSC to an MSC is the incorporation of data on variability. Such data are generally obtained by examination of herbarium specimens. For most European and North American species, such material is both sufficient and readily available. This is not true in the tropics, however, where many species are

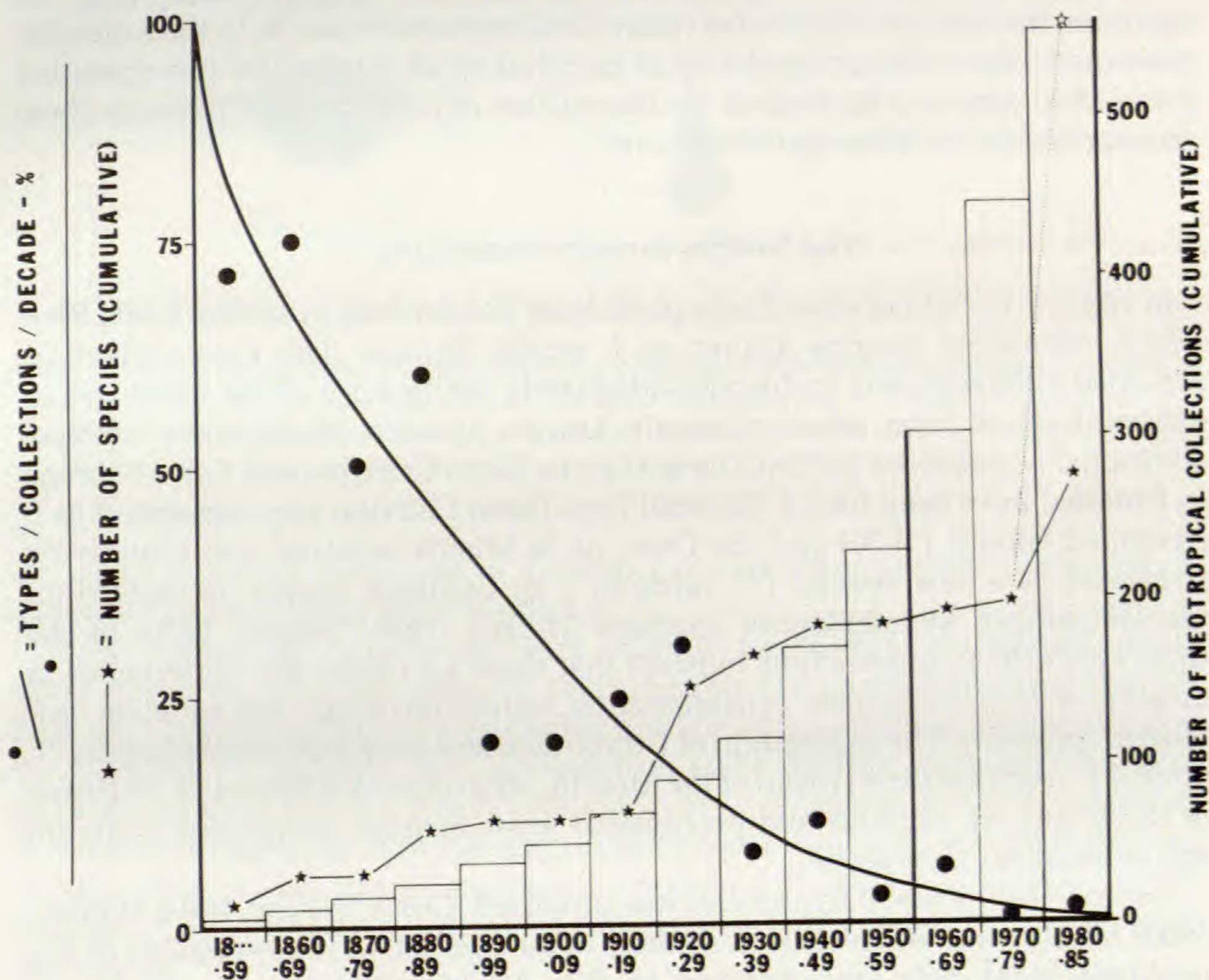


FIG. 1. Status of Neotropical *Isoëtes*. Bar graph indicates cumulative numbers of herbarium collections available for study, by decade, as of 1985; darker separation at 1960 indicates median of collections. Solid circles represent the number of type collections (%) obtained within the respective decades; dark solid line represents a "best-fit" approximation of trend. Solid stars represent the number of valid (*sensu* Hickey, 1985) species obtained as of the end of the respective decade; hollow star in the 1980-decade represents the alternative estimate of Fuchs-Eckert (1982).

represented by 3 or 4 specimens and a large number are still represented by only a single known collection. In the Neotropics this situation is gradually changing. Since 1960, the total number of Neotropical *Isoetes* collections has grown from 226 to over 560, a 150 + % increase in the last 37 years relative to the previous 120 (Fig. 1). An analysis of these collections (Fig. 1) suggests that we are approaching (in the Andes at least) an end to the collecting of new (morphological) species.

These herbarium collections represent the major resource for the development of the morphological species concept in *Isoetes*. Before 1960, for example, the average number of collections for Neotropical species was four; today that average is 12. Because the actual number of collections per taxon is usually higher than this (many Brazilian species are still only known from the type collection), estimates on interspecific variability can be made for most of them. As a direct result, we are now able to make reasonable hypotheses relative to species delimitations for many Neotropical species. The formation of alternative hypotheses regarding the delimitation of these species, such as can be seen when comparing the works of Fuchs-Eckert (1982) and Hickey (1985) (Fig. 1), represent the base line models for future biosystematic research. In the examples presented below, the accumulation of morphological data and its incorporation into a MSC were crucial steps in the elucidation of our current hypothesis about an appropriate working species concept.

THE ISOËTES STORKII AGGREGATE

In 1931, T. C. Palmer described a plant from Volcán Poás in central Costa Rica which ultimately became known as *I. storkii* Palmer. The type collection provided sufficient data to describe adequately the features of the plant and to differentiate it from other currently known species. Since then, several additional populations (at Cerro de la Muerte, Cerro Chirripó, and Cerro Fabrega in Panama) have been found. Material from Cerro Chirripó was segregated as *I. tryoniana* Gómez (1970) and the Cerro de la Muerte material was tentatively suggested as a new species ("I. mickelii") by Lellinger (based on herbarium identifications). Morphological analyses (Hickey, 1985; Gómez, 1970) of the three Costa Rican populations indicate that there are observable differences in megaspore size, megaspore ornamentation, velum coverage, leaf number, leaf length, and habit. The segregation of *I. tryoniana* and proposed segregation of "I. mickelii" were the direct result of the identification of such differences. All three of these taxa are diploids and presumably arose through geographic isolation and subsequent divergence.

A somewhat different hypothesis was proposed (Cox & Hickey, 1984; Hickey, 1985; Hickey, ms. submitted) as a result of an alternative interpretation of the morphological data (summarized in Fig. 2). Morphological variation in megaspore size (axis b), megaspore ornamentation (axis c), leaf number (axis d), and leaf length (axis e) were compared with differences in elevation (axis a). The inverse relationship between axis a and axes b–e suggests that the three populations represent a single species exhibiting clinal variation. Morphological variation among the populations was interpreted to be primarily

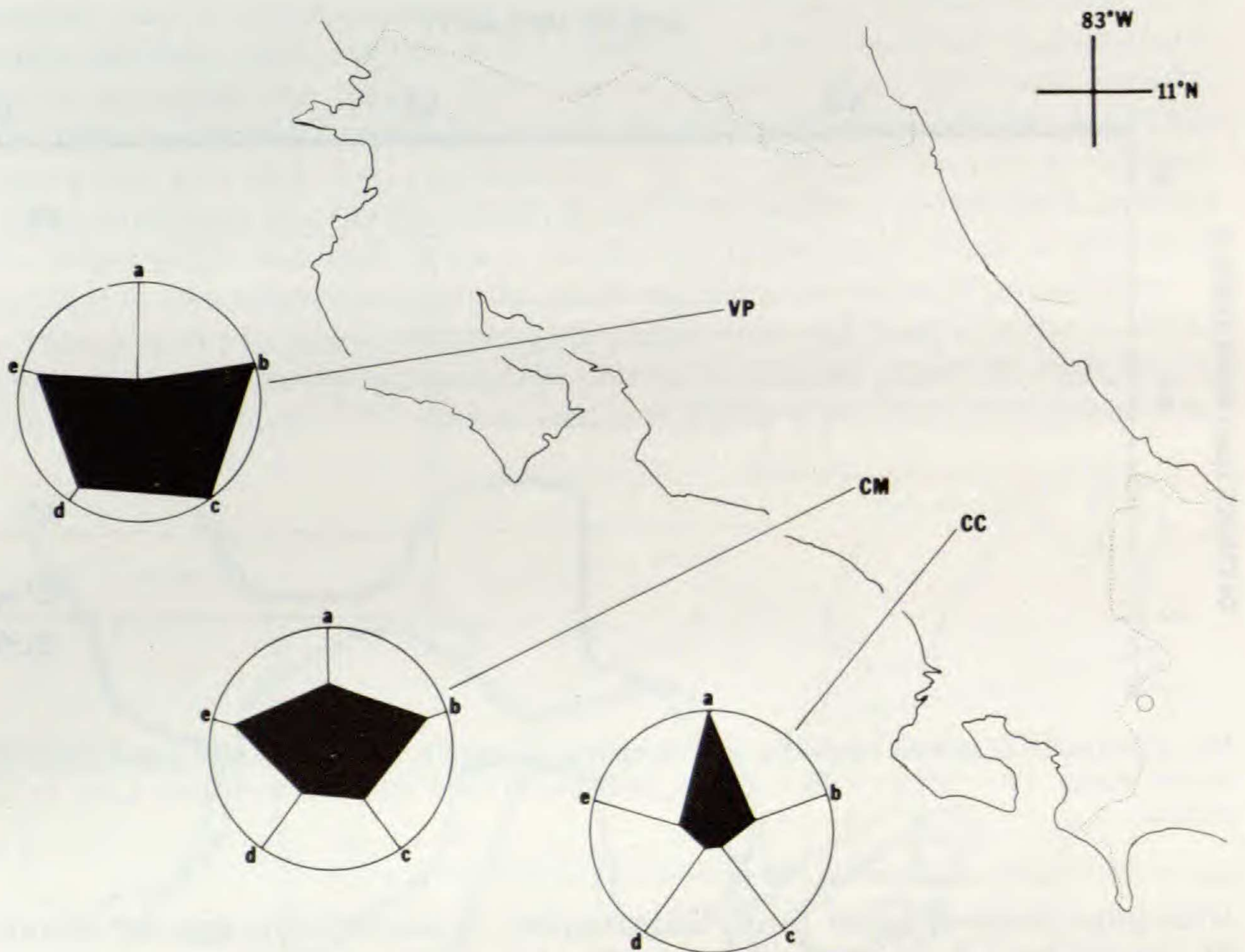


FIG. 2. Character polygonals for three Costa Rican populations of *Isoetes storkii*. Axis a = elevation (2500–3500 m); axis b = mean megaspore size (500–700 μm); axis c = megaspore ornamentation (slight, moderate, pronounced); axis d = mean leaf number (20–100); axis e = mean leaf length (0–100 mm). VP = Volcán Poás; CM = Cerro de la Muerte; CC = Cerro Chirripó.

the result of plastic responses to differences in elevation and the concomitant variations in temperature and insolation.

These competing hypotheses were recently tested (Hickey, unpubl. data) using starch gel electrophoresis on four presumptive populations from Volcán Poás and Cerro de la Muerte. Forty-three individual sporophytes were examined for variation at eighteen loci representing eight enzyme systems. The four populations were fixed for similar allozymes at 14 of these loci. Allelic variation in the four variable loci was low and overall genetic similarity was high (Fig. 3). Unlike other *Isoetes* species studied (*I. mexicana*, *I. cubana*, *I. pallida*, and *I. flaccida*; Hickey unpubl.) virtually no intrapopulation variation was detected in *I. storkii*.

The electrophoretic studies support the single species hypothesis of Hickey (1985) and suggest two models to account for the genetic characteristics of the populations studied. The first model hypothesizes that the Cerro de la Muerte and Volcán Poás populations were derived from a very few long distance dispersed spores; the paucity of genetic variation then is the result of an initial lack of genetic variability. A corollary to this model is the virtual lack of

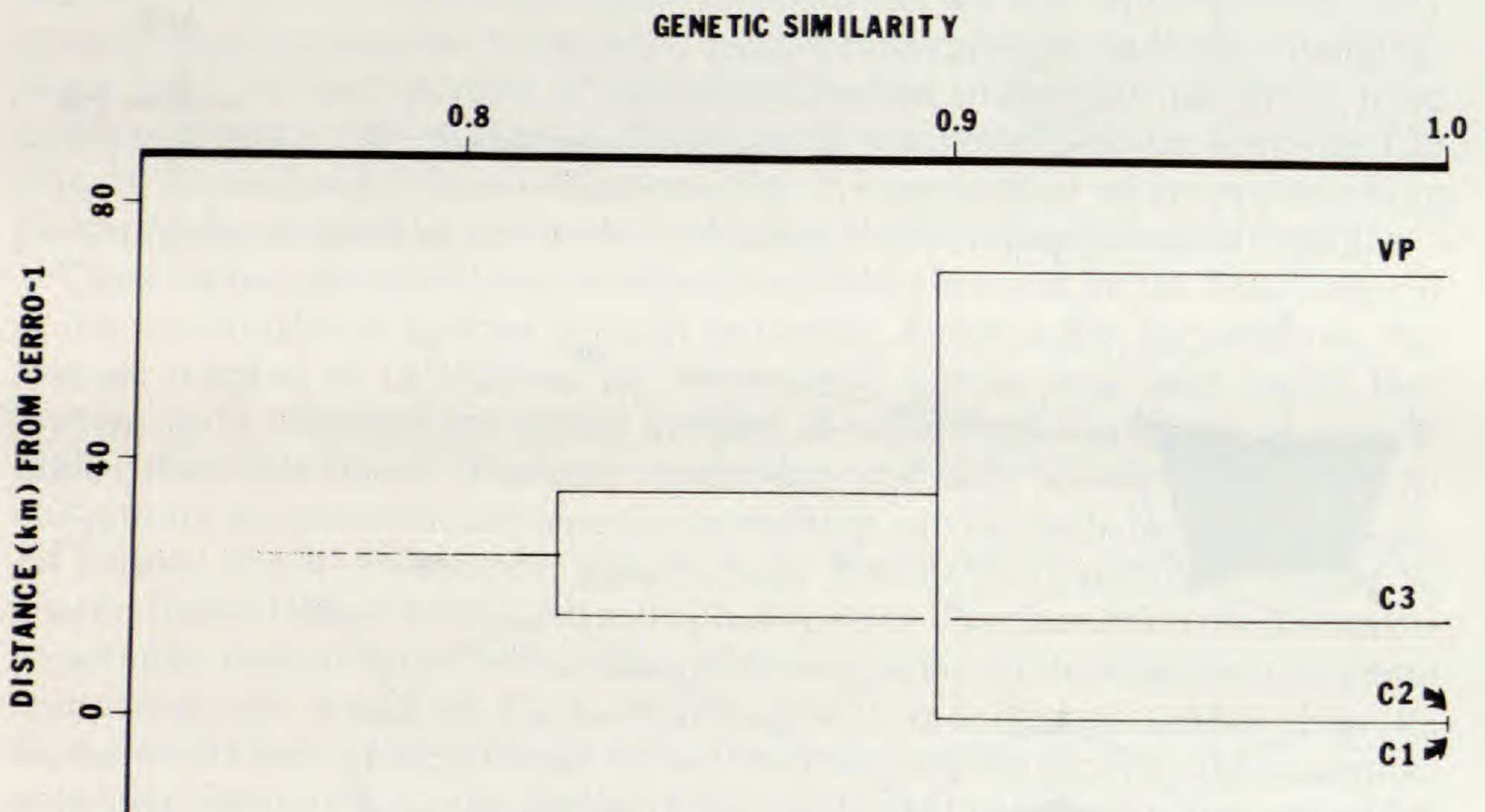


FIG. 3. Estimates of genetic similarity plotted against geographic distance for four populations of *Isoetes storkii*. VP = Volcán Poás; C1, C2 & C3 represent three subpopulations from Cerro de la Muerte.

interpopulational gene flow. This model is essentially one of island biogeography where the highest peaks of the Talamanca range represent discrete, ecological islands. Alternatively, the lack of genetic variability within and between populations may be stochastic and result from severe genetic bottlenecks. Vulcanism (Volcán Poás) and fires (Cerro de la Muerte) are likely probable causes for population fluctuations in these areas. Such bottlenecks could deplete genetic variation even in the face of limited introduction of additional propagules. These models, either alone or in concert, would account for the low genetic variation seen in the Cerro de la Muerte and Volcán Poás populations. Both scenarios suggest that the populations studied are peripheral, derivative populations and both predict the existence of a genetically more diverse source population at either Cerro Chirripó or Cerro Fabrega.

The morphological variation seen in the Costa Rican populations of *I. storkii* is believed to be primarily the result of an elevational phenocline, possibly supplemented by random genetic variation. Such interpopulational variation may reflect an initial stage in allopatric speciation (Tryon, 1986).

THE ISOËTES MELANOPODA COMPLEX

In the southeastern United States there are a number of diploid *Isoetes* which are endemic to granitic outcrops. These species are known, on the basis of enzymatic (Hickey et al., 1989), morphological, and phenological (Table 1; Fig. 4) synapomorphies, to be closely related to the more widespread *I. butleri*

Engelm. and *I. melanopoda* Gay & Durieu. All of these species are tropical in origin (Hickey, 1985; Hickey et al., 1989) and, while doubtfully holophyletic, are of monophyletic origin. With few exceptions, these taxa were initially characterized on the basis of one or at best a few collections and the transition from a TSC to a MSC was a gradual one. The morphological analyses of Boom (1979), Matthews and Murdy (1969), Reed (1965) and Rury (1978) have outlined the infraspecific variation in these plants. Not surprisingly, these studies have resulted in somewhat divergent opinions regarding the status of several taxa.

This complex is now undergoing electrophoretic and biosystematic analyses and, while the data are not complete, several trends are apparent. Using triose phosphate isomerase (TPI) as an example, it will be noted that phylogenetically

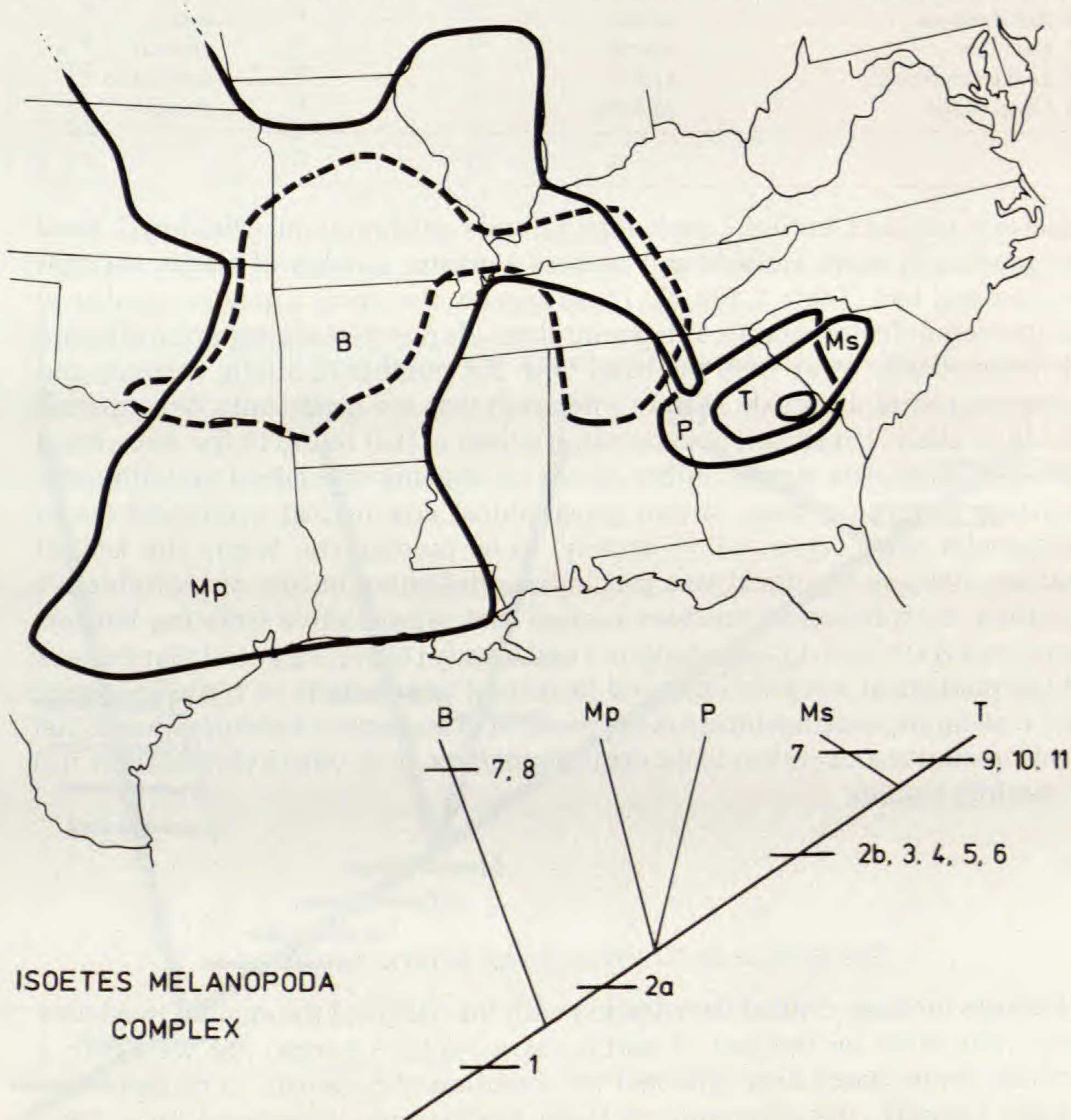


FIG. 4. Geographic distribution and phylogenetic reconstruction of the *Isoetes melanopoda* complex. Character numbers refer to those listed in Table 1. B = *I. butleri*; Mp = *I. melanopoda*; P = *I. piedmontana*; Ms = *I. melanospora*; T = *I. tegetiformans*.

TABLE 1. Polarized Morphological Character States Used for Phylogenetic Analysis of the *I. melanopoda* Alliance. Outgroups for polarization are those established by Hickey (1986) and the *I. panamensis* alliance.

Character	Pleisomorphic	Polarity	
			Apomorphic
1. Phenology	fall		spring
2. Velum coverage	absent	partial —	complete
3. Fibrous bundles	present		absent
4. Leaf X-section	trigonal		+/-terete
5. Megaspore color	white		brown-black
6. Scales/Phyllopodia	present		absent
7. Microspore surface	echinate		papillate
8. Substrate pH	acidic		basic
9. Gemmae	absent		present
10. Leaf arrangement	spiral		distichous
11. Corm shape	globose		elongate

basal taxa such as *I. butleri*, *I. melanopoda*, and *I. piedmontana* (Pfeiffer) C. Reed are genetically more variable and possess a greater number of allelic variants over several loci (Table 2, Fig. 5). These species also show a greater number of accumulated electrophoretic autapomorphies. As one moves geographically and phylogenetically away from the basal taxa, the number of allelic variants and autapomorphies diminish. Distal (= derived) taxa are genetically depauperate and their allelic constitution represents a subset of that found in the more basal species. These data suggest either recent or ongoing speciation via allopatry. Isolation among the taxa, in part geographical and in part ecological (as in *Selaginella* spp.; Tryon, 1971), appears to be incomplete. While the lack of marker alleles in the distal taxa precludes a definitive testing of reproductive isolation, morphological analyses suggest that reproductive isolating barriers have not yet evolved. In fact, Matthews and Murdy (1969) suggested that the lack of morphological integrity observed in several populations of *I. piedmontana* and *I. melanospora* Engelman is the result of introgressive hybridization. Final confirmation regarding this isolation is dependent on in vitro hybridization and F_1 sterility studies.

THE ISOËTES OF NORTHEASTERN NORTH AMERICA

Perhaps the most critical data dealing with the nature of the species in *Isoëtes* came from work on the taxa of northeastern North America. For well over a century, these plants have attracted the attention of botanists working in this region. Initially, the taxonomy of these *Isoëtes* was influenced by a TSC, resulting in over thirty published species, varieties, and forms. However, the last MSC account of this region accepts only eight species and no infraspecific taxa (Kott & Britton, 1983). More recently, the application of experimental techniques

TABLE 2. Distribution of TPI Alleles within the *Isoëtes melanopoda* Complex.

Character Number	Locus	Allele	Species				
			B	Mp	P	Ms	T
1.	I	A		+			
2.	I	B			+		
3.	I	C	+	+	+	+	+
4.	I	D	+				
5.	I	E	+	+			
6.	II	A		+	+	+	
7.	II	B		+	+	+	+
8.	II	C			+		
9.	II	D	+				
10.	II	E		+	+	+	
11.	II	F	+				
12.	II	G		+	+		
13.	II	H	+				

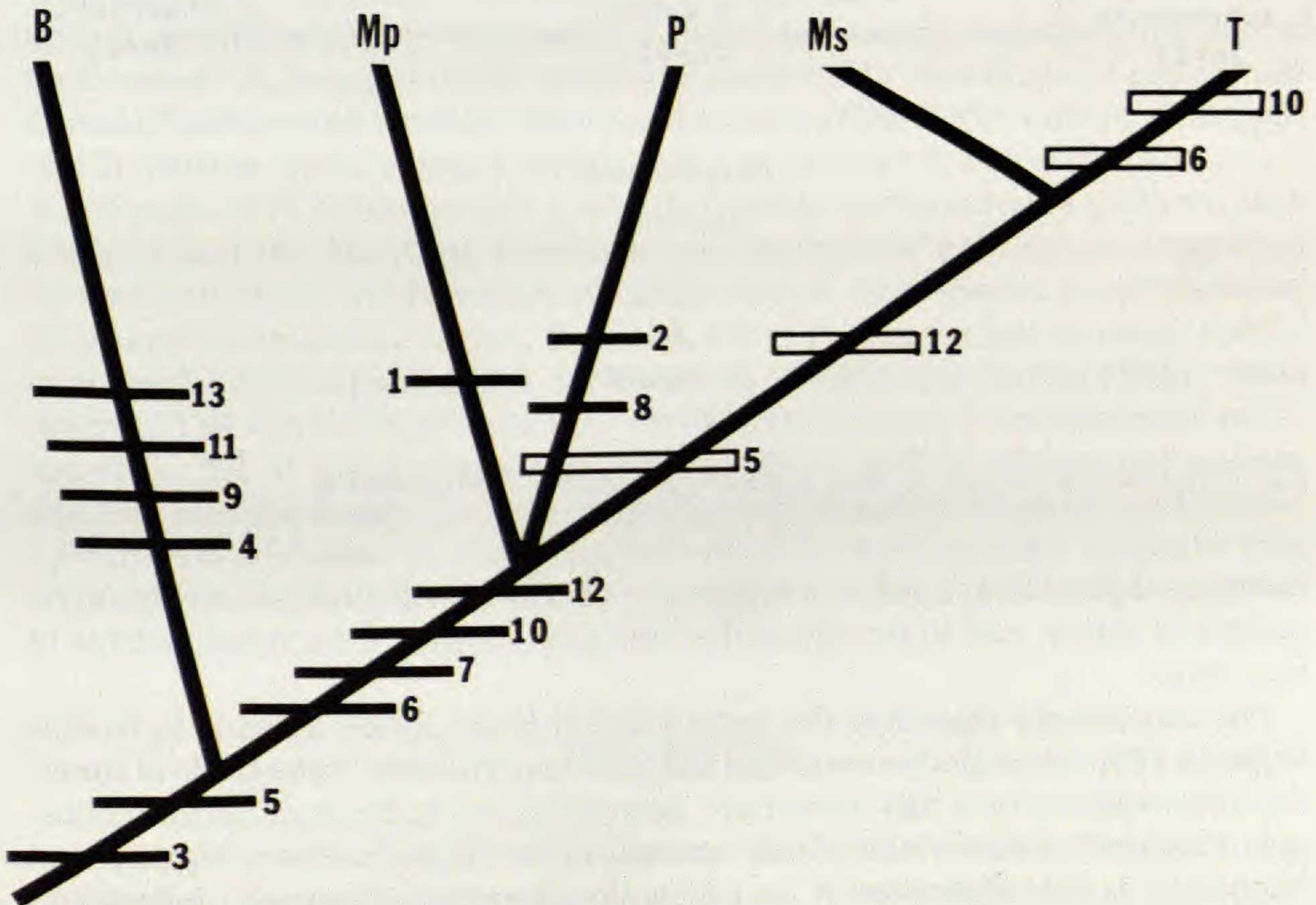


FIG. 5. Overlay of TPI allele distribution onto cladogram in Fig. 4. One of several equally parsimonious reconstructions; this assessment favoring allele loss over convergence. Data as in Table 2.

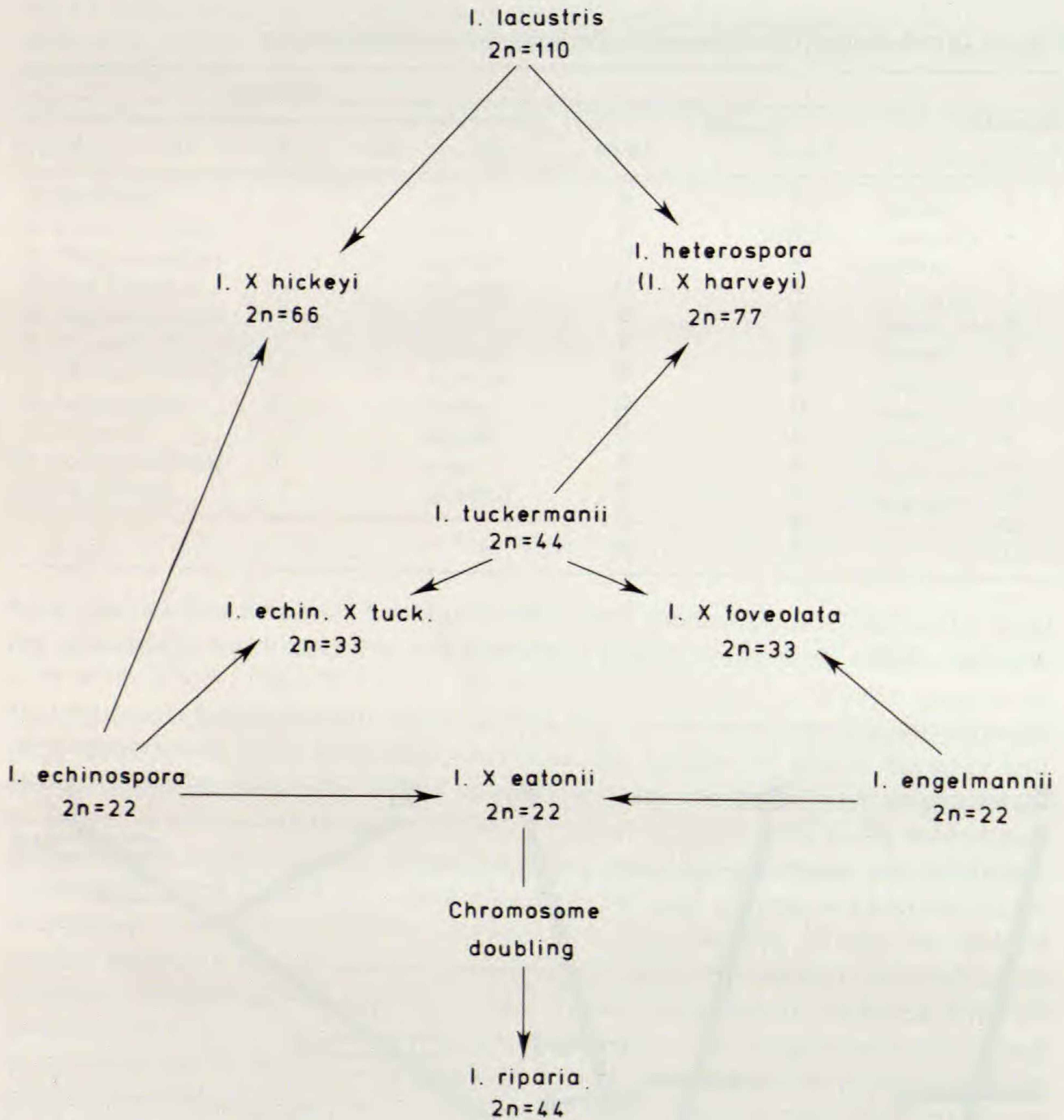


FIG. 6. Hypothetical pedigree for some *Isoetes* in northeastern North America.

has made it possible to confirm the presence of interspecific hybrids, to identify a polyploid series, and to recognize the reticulate nature of the relationships of these taxa.

The controversy regarding the occurrence of interspecific hybrids in *Isoetes* began in 1896 when Dodge remarked that species of *Isoetes* "intergrade at times, *Isoetes tuckermanii*, for instance, appearing to hybridize with *Isoetes echinospora*." Eaton (1900) took exception to Dodge's claim stating that "hybridity is extremely rare, if not altogether absent in the genus." Jeffrey and Hicks (1925) and Jeffrey (1937) reported on a quillwort from Nova Scotia that produced megaspores that varied tremendously in size and shape, microspores that were mostly empty, and meiotic cells that showed lagging chromosomes

during microsporogenesis. They attributed these phenomena to interspecific hybridization. Kott and Britton (1983) maintained that there was insufficient evidence to state that hybridization occurs naturally among the *Isoëtes* of northeastern North America. They asserted that either ethological or sterility barriers exist among most taxa.

The first experimental data pertinent to these conflicting views came from Boom (1980), who reported successful, artificial crosses between four species of *Isoëtes*. The ease with which hybrids formed in culture suggested to Boom that hybridization followed by polyploidization was a feasible mode of evolution in the genus. Thus, Boom's work provided a rational, testable hypothesis regarding allopolyploid speciation in *Isoëtes*.

Evidence from spore morphology, chromosome number, enzyme electrophoresis, and in vitro germination and hybridization experiments was provided by Taylor et al. (1985) to support the hypothesis that sterile, interspecific hybrids occur in nature and that some of these diploids could have been involved in the evolution of fertile allopolyploid species. Specifically they proposed that *I. echinospora* Durieu, a circumboreal diploid and *I. engelmannii* A. Braun, an eastern North American diploid, hybridized to form *I. × eatonii* Dodge (pro sp.), a sterile, diploid plant occurring mainly within the overlapping range of the putative parents. In addition, they suggested that *I. riparia* A. Braun, a fertile tetraploid distributed along the eastern seaboard, is an allotetraploid derivative of *I. × eatonii*. Their data also indicate that *I. × foveolata* A. Eaton (pro sp.), a sterile triploid, represents a cross between *I. engelmannii* and *I. tuckermanii* A. Braun, a fertile tetraploid restricted to New England and eastern Canada. More recent work by Taylor and Luebke (1986, 1987, and unpubl. data) and D. Britton (pers. comm.) reveals that *I. × harveyi* A. Eaton (pro sp.) and *I. × heterospora* A. Eaton (pro sp.), both commonly encountered in northern New England and the Maritime Provinces, are heptaploid hybrids resulting from crosses between *I. tuckermanii* A. Braun and *I. lacustris* L., a circumboreal decaploid. In this same region, they also have identified a less common hybrid between *I. echinospora* and *I. tuckermanii*. Luebke and Taylor (1985, 1988) reported the discovery of interspecific hybrids between *I. echinospora* and *I. lacustris*. All of these putative hybrids produce aborted spores and possess additive electrophoretic profiles. The thorough documentation of interspecific hybrids in addition to the presence of polyploid species indicate that allopolyploid speciation has been an important factor in the evolution of *Isoëtes* in northeastern North America (Fig. 6).

CONCLUSIONS

The formulation of a working species concept in any genus is an historical, stepwise process. In many animal groups and most definitely in *Isoëtes*, the progression has been from a typological to a morphological to a biological species concept. The transitions between these steps are the result of differing objectives and methodologies, each of which contributes unique insights toward an understanding of the various species involved.

The TSC-MSB transition is highly dependent upon significant numbers of collections and an analysis of variation. As discussed above, this transition is still far from complete for tropical *Isoetes* but the collections of D. Breedlove, A. Cleef, J. Cuatrecasas, S. and J. Keeley, B. Øllgaard, and J. Steyermark, for example, have made such a transition possible for at least some parts of the Neotropics. While the primary focus and importance of this transition is in the establishment of workable taxa, the accumulated data often form the hypotheses and needed background for the MSB-BSC transition. This is shown particularly well by the work in the *I. storkii* analyses and in the ongoing work with the *I. melanopoda* complex.

The establishment of the biological species concept for *Isoetes* is a recent development. In part, this has been because its establishment is inseparable from an understanding of the nature and modes of speciation (Tryon, 1986). In *Isoetes*, we are just beginning to investigate and understand these mechanisms. However, the data accumulated to date are compelling. The works of Boom (1980) and Taylor et al. (1985) have clearly shown that pre-zygotic isolating mechanisms do not exist between aquatic species in eastern North America and that natural interspecific hybrids occur in areas of sympatry.

The works of Hickey, Luebke, and Taylor indicate that allopatric divergence may well be the initial mode of speciation in the genus. Spatially isolated, divergent taxa produce sterile F_1 hybrids upon subsequent contact suggesting that post-zygotic isolating mechanisms form rapidly. A 56% incidence of polyploidy in the genus (Hickey, 1984, and unpubl. data) indicates that the results of Taylor and Luebke's work on the species of northeastern North America may be typical of the genus as a whole. That is, polyploidization is a frequent associate of hybridization and that allopolyploidy may be a significant, secondary speciation mechanism in *Isoetes*.

In conclusion, the data currently available suggest that species of *Isoetes* appear to have evolved in two ways. First, species have evolved gradually as the result of spatial isolation and genetic divergence; second, species have formed abruptly by interspecific hybridization followed by chromosome doubling. Available data also suggest that the genus is currently undergoing rapid and continuing speciation (both divergent and abrupt), a situation that often confuses taxonomic boundaries under the restraints of a morphological (or any other non-dynamic) species concept. All of the species of *Isoetes* that have been intensively investigated readily conform to a dynamic interpretation of the biological species concept. With additional study, our species concept, interpreted as a working hypothesis, will undoubtedly change.

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